

The Embryology of *Garcinia mangostana* L. (Clusiaceae)

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Abstract

The anther is tetrasporangiate. The anther wall is four-layered and the secretory tapetum is binucleate. Most of the pollen mother cells degenerate just before meiosis and a few undergo meiosis. Cytokinesis is simultaneous and tetrads are tetrahedral.

The ovule is anatropous, bitegmic, tenuinucellate and the micropyle is formed by the outer integument only. The embryo sac development is of the *Polygonum* type.

The endosperm is of the Nuclear type. Cell-wall formation proceeds from the periphery towards the centre. The development of the adventive integumentary embryos is irregular and sometimes within the same embryo sac, several embryos may be found. The mature embryo is straight. The seed coat develops from the outer integument. The seed is non-endospermous and shows hypogeal germination and 10% of the germinated seeds give multiple seedlings.

Introduction

The mangosteen (*Garcinia mangostana* L.) commonly known in Malaysia as the "Queen of fruits" is indigenous to the Malay Archipelago (Burkill, 1935) but the present distribution stretches from southern India through the Malesian region to as far as the Philippines. The plant is dioecious, of medium height (5–15 m) and produces a characteristic opaque, yellow latex. It is slow-growing and requires approximately 15 years to bear fruits. In Peninsular Malaya, the mangosteen produces fruits twice a year; between June and August, and October and December. This seasonality can easily be upset by any change in the pattern of the wet and dry seasons. Most individual trees, however produce fruits only once a year, some during the first season and the others during the second. The fruits are usually eaten fresh or they can be made into a pleasant preserve called "halwa manggis".

As early as 1919, Sprecher described the fruits, seeds and the embryogeny of *G. mangostana*. Treub (1911), Puri (1939), Horn (1940) and much later Ha (1978) contributed some information on the flower, fruit and seed development in *Garcinia*. Nevertheless, our knowledge of the embryology on this genus remains fragmentary.

Materials and Methods

Buds, flowers and fruits of *G. mangostana* were collected at regular weekly intervals from the fruit-tree nursery, Ministry of Agriculture, Serdang, West Malaysia. A voucher specimen KLU 1837 was deposited in the Herbarium, Botany Department of the University of Malaya. The buds and flowers were fixed in formalin-propionic-alcohol (5 c.c. formalin, 5 c.c. propionic acid and 90 c.c. 50% ethanol). For fruits, the fixing solution used was Craff III (30 c.c. 1% chromic acid, 20 c.c. 10% acetic acid, 10 c.c. 37% formaldehyde and 40 c.c. distilled water) at 0°C, as 50% F.A.A.

hardened them excessively. Customary methods of dehydration and embedding were followed. Much difficulty was encountered in sectioning the fruit tissues because of the bulk and presence of sclerenchyma tissue. To overcome this, the wax blocks, with the tissues exposed, were immersed for a month in a softening solution of Molifex (B.D.H., U.K. Co.) or Bakers solution (36 c.c distilled water, 54 c.c. 95% ethanol, 10 c.c. glycerol). Microtome sections were cut at 12μ and stained in 1% alcoholic (in 50% alcohol) safranin and 0.5% alcoholic (in 95% alcohol) fast green FCF.

Observations and Results

FLORAL AND FRUIT MORPHOLOGY

The flowers about 4 cm wide, borne singly or in pairs, are terminal in position. The four curved sepals are persistent on the fruit and the four fleshy petals are tinged red (fig. 1A-C). The superior ovary is round, capped by the sessile 5-8-lobed stigma. The base of the ovary is surrounded by 14-16 staminodes. Anthesis is in the evening between 4 and 6 pm and after 24 hours the petals drop off whereas the sepals persist on the ripe fruit.

The fruits are approximately 8 cm in diameter and when ripe, have a purplish rind 0.5 cm thick. Within the rind, there are 5-8 fleshy or pulpy segments which, in some, contain a light, brown seed that adheres to the flesh. The texture of the pulp is soft, delicately flavoured and slightly acidic in taste.

The mature seeds are non-endospermous, flattened and of various sizes. They are usually 1.0-1.5 cm long and 0.3-0.5 cm thick.

MICROSPORANGIUM, MICROSPOROGENESIS AND MICROGAMETOPHYTE

The anther is tetrasporangiate with four distinct wall layers within the epidermis *viz.*, the endothecium, two middle layers and the tapetum (fig. 2A-D). The secretory tapetum is initially uninucleate but later becomes binucleate (fig. 2C). It degenerates soon after the formation of microspore tetrads.

In young anthers, the microspore mother cells are well formed and prominent (fig. 2B, 2C). However, as they enter into the meiotic division, their nuclei disintegrate first and then their cytoplasm, causing most of them to degenerate (fig. 2K). A few do undergo reduction division and simultaneous cytokinesis (fig. 2F-J) but the chromosomes stain very faintly and show signs of disintegration. At various stages of meiosis, the microspore mother cells continue to degenerate in large numbers. A few microspore tetrads and single microspores appear normal but the latter were non-viable as shown by their failure to stain in 1% 2, 3, 5-triphenyl tetrazolium chloride.

Simultaneous with the degeneration of the tapetal cells, the epidermis accumulates tanniniferous materials while fibrous thickenings are formed in the endothecium and the middle layers (fig. 2E) and all these persist in the mature flower.

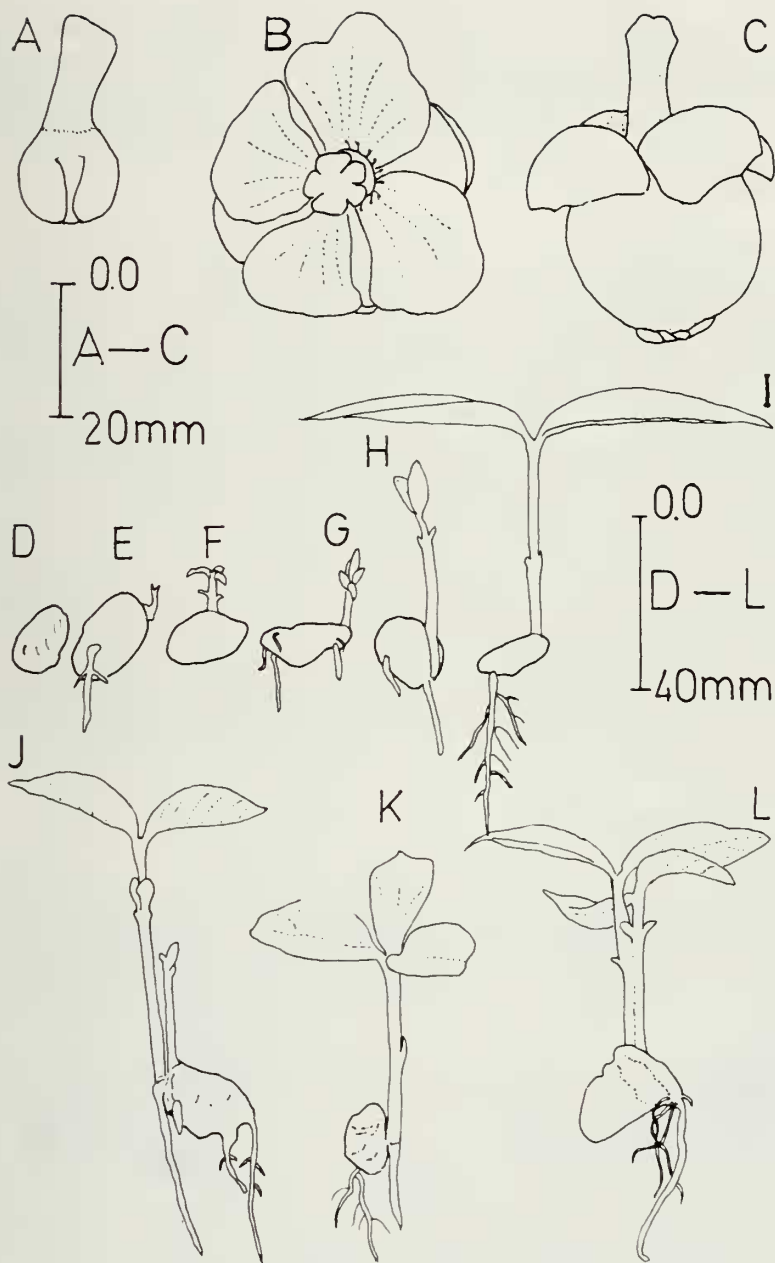


Fig. 1. Flower, fruit and seedling morphology. *A*, flower bud; *B*, flower; *C*, fruit; *D-I*, seed germination; *J*, polyembryonic seedling; *K*, "three-leaved" seedling; *L*, twin seedlings partially fused along the stem.

MEGASPORANGIUM, MEGASPOROGENESIS AND MEGAGAMETOPHYTE

The ovular primordium develops from the placenta. The differentiation of the inner integument is followed by that of the outer (fig. 3A-C). Simultaneously, one of the hypodermal, nucellar cells enlarges to form the archesporial cell (fig. 3A, 3B). The ovule is anatropous, bitegmic, tenuinucellate and the micropyle is formed by the outer integument only (fig. 3K). A short, thick funiculus is present. Only one ovule is present within each loculus.

The archesporial cell enlarges to function directly as the megaspore mother cell (fig. 3A, 3B). At this stage, the outer integument gradually outgrows the inner, enclosing the nucellus to form the micropyle (fig. 3C). Usually only one archesporial cell is produced but sometimes two or three are formed. Following the first meiotic division in the megaspore mother cell, a pair of unequal dyad cells is developed and the pair divides further to form a linear tetrad. At the completion of meiosis, only the chalazal megaspore enlarges further to form the female gametophyte while the other three megaspores degenerate (fig. 3F). The nucleus of the functional megaspore undergoes three successive divisions to form an 8-nucleate embryo sac which is of the *Polygonum* type (fig. 3H-J). The antipodals usually degenerate soon after formation but rarely do they persist even up to the stage when the egg apparatus degenerates (fig. 4A). Approximately 50% of the ovules examined showed degeneration of its embryonic content (fig. 3K). This was observed during various stages of the embryo-sac formation. Often, the multiple archesporial cells differentiate into the megaspore mother cells and further develop into multiple embryo sacs. The development of multiple embryo sacs is not synchronous and frequently, all but one degenerate (fig. 3D-G).

POLLINATION AND FERTILISATION

In the female flower, viable pollen grains are never found and those formed are empty and non-viable. Viable pollen grains are probably produced in the male flower but there is no report of a male *G. mangostana* tree in the Malay Peninsula. Parthenocarpy and apomixis in the species had been suggested by Corner (1952).

In order to confirm this inference, bagging experiments were done. A set of 100 flower buds (diameter 1.5 cm) at similar stages of development was selected and tagged. While 50 were bagged with the anthers removed, the rest were bagged with the anthers intact. Fruit set in the samples bagged with anthers removed versus anthers intact were 20% and 53% respectively. This suggests that cross pollination and fertilisation are not necessary for fruit formation. The removal of anthers from the buds before anthesis also excludes the possibility of self pollination. The percentage of fruit set in flowers with intact stamens is much higher than that with the anthers removed, possibly because of the unavoidable injury while emasculating.

ENDOSPERM

Before the degeneration of the synergids, the two polar nuclei fuse to form a secondary nucleus. The division of this is not followed by wall formation and hence

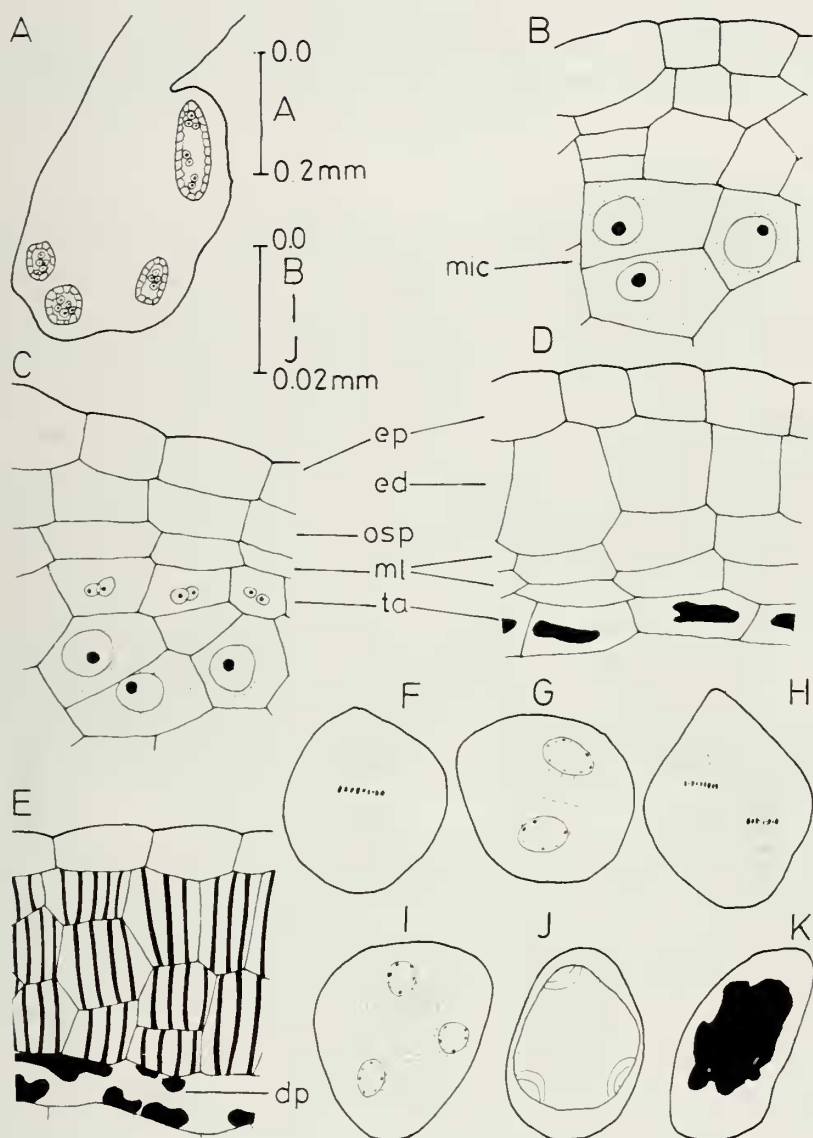


Fig. 2. Microsporangium and microsporogenesis. (*dp*, degenerated microspore mother cell; *ed*, endothecium; *ep*, epidermis; *mic*, microspore mother cell; *ml*, middle layer; *osp*, outer secondary parietal layer; *ta*, tapetum). *A*, tetrasporangiate anther; *B-E*, stages of anther wall development; *F-J*, meiosis in microspore mother cell; *K*, degenerated microspore mother cell.

endosperm formation is of the *ab initio* Nuclear type. The embryo sac enlarges at the expense of the nucellus and the inner integument. Free nuclei increase in number and remain scattered within the enlarging embryo sac (fig. 4B-D). Proceeding from the periphery to the centre, they become cellular when the embryo is in its advanced stage of development. This endosperm is progressively consumed by the developing embryo and in a fully mature seed, only one or two layers of it remain.

EMBRYO

The degeneration of the egg apparatus follows that of the antipodals and the fusion of the polar nuclei. However, one instance of an egg (probably developing into an embryo) surrounded by endosperm nuclei was observed (fig. 4F). An exceptional case of a persistent antipodal, was also observed simulating a developing egg (fig. 4A). As the embryo sac enlarges, the nucellus disintegrates, leaving only one or two layers of cells in the chalazal region. The degeneration of the nucellar tissue is followed by that of the inner integument, leaving only one or two layers of cells at the chalazal region of the embryo sac. When the embryo sac is about 0.4 cm long, the cells of the inner integument will have completely degenerated and the innermost layer of the outer integument becomes meristematic and divides rapidly. Undulations appear along the inner wall of the embryo sac. Cells of the outer integument divide periclinally and anticlinally, producing buds which project into the embryo sac. The divisions in these buds are irregular and buds of different shapes result *vis.*, button-like, linear etc. (fig. 4G-L). In the early stages of development, numerous integumentary buds are formed, but as development proceeds only a few of these attain maturity. Both the linear and button-shaped 'proembryos' eventually form broad-based, irregularly shaped embryos (fig. 4P). The developing embryos apparently obtain nutrients mainly from the integument and the nuclear endosperm.

The two cotyledons of mature embryos differ in size and shape. The epidermal cells of the cotyledons are small and their nuclei prominent while all the other layers have large and vacuolated cells with their nuclei peripheral. Polyembryony occurs when more than one bud matures within an embryo sac. In 50% of the ovules examined, the content of the embryo sac degenerates but the development of seed coat and ovary wall proceeds normally (fig. 3K). This produces seedless fleshy segments.

SEED COAT

In a mature ovule, the inner and outer integuments are respectively two layers and five to six layers thick (fig. 4M). At the time when the outer integument becomes meristematic, the outer epidermis of the outer integument accumulates tannin (fig. 4N). As development proceeds, more tannin is deposited in the cells of the outer integument. At the mature embryo stage, the enlarged embryo-sac cavity is lined by one or two layers of parenchyma cells while the rest of the cells of the outer integument are interspersed with tanniniferous cells (fig. 4O).

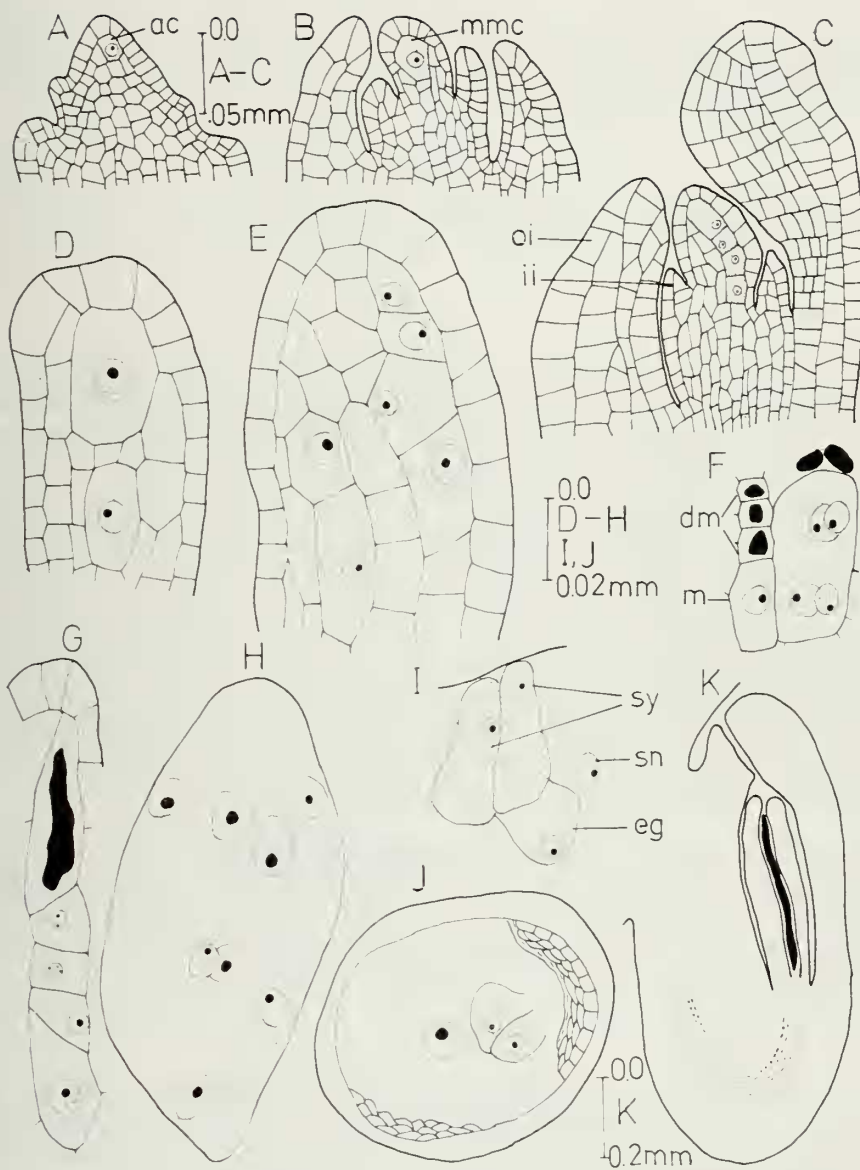


Fig. 3. Megasporangium, megasporogenesis and megagametophyte (*ac*, archesporium; *dm*, degenerating megaspore; *eg*, egg; *ii*, inner integument, *m*, megaspore; *mmc* megaspore mother cell; *oi*, outer integument; *sn*, secondary endosperm nucleus; *sy*, synergid). A-C, development of integuments; D-G, development of twin embryo sacs within an ovule; H, a developing embryo sac; I, egg apparatus with secondary endosperm nucleus; J, *t.s.* embryo sac showing the egg, one synergid and secondary endosperm nucleus; K, degeneration of embryonic content within an ovule.

FRUITS AND SEEDS

When the fruit fully mature, the diameter varies from 5 to 7 cm. The number of segments (5–8) within it corresponds to the number of stigmatic lobes of the flower. The fleshy segment may enclose a seed or it may be seedless. A random count of fifty fruits showed that the ratio of seeded to seedless segments was 1:2.

The seeds are of various sizes ranging from 0.5 to 2.0 cm in width. They are brown, flattened, have no endosperm and traversing its surface is a network of vascular bundles.

SEED GERMINATION

Seed germination is hypogeal and the incubation period is one week. The viability of fresh seed is very high, showing an average of 91% germination. During germination, the testa splits, one small root emerges from one end of the seed a few days before the appearance of the plumule at the opposite end (fig. 1D-G). The erect shoot system grows to about 4.0 cm before unfolding its first pair of leaves (fig. 1H-I). Normally, only one seedling germinates from each seed but in approximately 10% of the seeds germinated, polyembryony occurs and commonly, two or three, as many as even four seedlings per seed have been observed. All these seedlings arise from different positions of the seed and possess their own separate root systems (fig. 1J). Occasionally, the two to three shoots may arise from the same point of the seed and then they share one root system. This occurs as a result of the development of lateral buds in the shoot as can be seen from the longitudinal section of the multiple shoots which shows that the vascular bundles of the shoots are branches of the main vascular bundle of the hypocotyl (fig. 4Q).

Fasciation is another common phenomenon among the seedlings. Often two seedlings are partially fused along the stem or the two shoot systems are completely fused except at the apices (fig. 1L). Sometimes, a seedling may have three leaves at a node owing to incomplete leaf separation (fig. 1K).

Discussion

In *G. mangostana*, so far, in Malaya only trees with female flowers are known in cultivation. According to Corner (1952), "the male trees have never been found in Malaya though they are said to occur rarely in Indochina". In addition, fertile anthers are not found in the female flowers. Chevalier (Bull. Agric. Instit. Sci. Saigon, I, 1919 pg. 101) in Burkill (1935) stated that the female plant produced flowers which had male organs and that one could suppress all the male plants in a garden without affecting fruit set.

Within the family Clusiaceae, the development of pollen grains has not been investigated (Davis, 1966). This present study shows that the anther of *G. mangostana* is tetrasporangiate and degeneration in female flowers has been observed at various stages of microsporogenesis. In addition, the few microspores produced were tested and found non-viable. This observation strengthens the earlier report of Corner

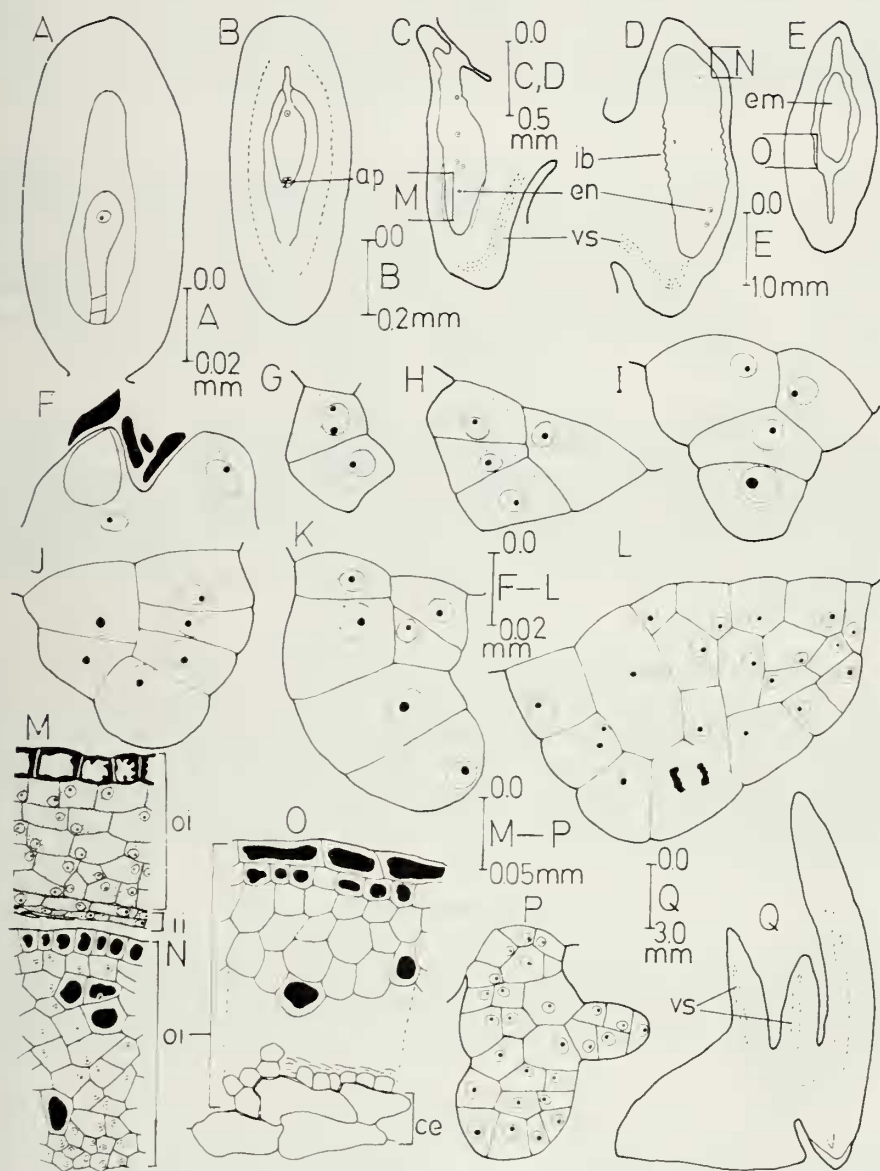


Fig. 4. Embryogeny and development of the seed coat. (*ap*, antipodal; *ce*, cellular endosperm; *em*, embryo; *en*, endosperm nucleus; *ib*, integumentary buds; *ii*, inner integument; *oi*, outer integument; *vs*, vascular system). A, persistent antipodal developing into embryo; B-E, l.s. ovule showing seed coat development; F, egg with endosperm nuclei, G-L, development of integumentary embryo; M-O, portions of seed coat enlarged from C-E; P, an irregularly shaped embryo; Q, germinated seed with multiple shoots.

(1952) and suggests apomixis as the mode of reproduction. Within the genus *Garcinia* pollen-grain degeneration has also been reported in the staminodes of the female flowers of *G. livingstonii* (Puri, 1939) whereas in *G. forbesii*, the microspore mother cells do undergo simultaneous cytokinesis to produce binucleate, mature pollen grains (Ha, 1978).

Preliminary bagging experiments confirm that the seeds are formed without fertilization. However, the fruits formed from bagged flowers with the anthers removed are slightly smaller (diameter 4.0 cm) than those with the stamens intact (diameter 4.8 cm). This is probably due to physical damage during the process of removing the anthers. Similarly, Maguire (1976) found that some *Clusia* species occurring in the semi-deciduous forests in Guayana also produced fruits from unfertilized flowers. But he did not establish the type of asexual reproduction.

The present study reaffirms the early observation of adventive embryony in *G. mangostana* by Sprecher (1919). Horn (1940) further explained that since all the seedlings were derived from essentially the same type of cells i.e., the integument, they were of exactly the same genotype. That explains that hence throughout Malaya, the fruits of *G. mangostana* taste alike and there is only one variety present. A race with a thicker rind and more acid flesh is said to occur in the Sulu Islands (Burkill, 1935).

Endosperm formation in the Clusiaceae is Nuclear in type and embryogeny conforms to the Onagrad type (Davis, 1966). In *G. mangostana*, the actual type of embryogeny could not be determined because of the irregular sequence of cell division in the integumentary buds. The various species of *Garcinia* in particular and the members within the Clusiaceae in general should be investigated extensively in order to establish the embryological pattern of development in the family.

Acknowledgements

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